Examination of the Gelatin Degradation Products by Gelatinase B using Synchrotron Infrared Microspectroscopy

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Matrix metalloproteinases are a family of enzymes that degrade components of the extracellular matrix in processes that demand tissue remodeling. One of the members of this family is gelatinase B. This enzyme degrades gelatin, one of the components of extracellular matrix and basement membranes. We report on the application of infrared microspectroscopy to probe the degradation of gelatin, collagen, and matrigel fibers by human neutrophil gelatinase B. In this work, matrigel films, which are a model system for the extracellular matrix in tissues, are prepared on BaF2 surfaces. A small amount of gelatinase B enzyme is added and reacted with the film for 12 hours in an incubator at 37 degrees C. The structure of the degraded products is examined by infrared micro-spectroscopy of the Amide I (protein) band. A comparison of the infrared spectra of degraded and non-degraded gelatin shows a dramatic shift and broadening of the Amide I component in degraded matrigel (**Figure 1**). By mapping the area of degraded matrigel, results show that the enzymatic reaction advances along the edges of the matrigel fiber.

Similar work is being performed on gelatin films. Gelatin is labeled with fluorescein, and thin films are prepared on BaF2 surfaces. Green gelatin fibers are visible using a fluorescence microscope. A small amount of gelatinase B enzyme is added and reacted with the film for 12 hours in an incubator at 37 degrees C. Gelatin degradation by the enzyme is observed visibly by the loss of green fluorescence on the BaF2 surface. The structure of the degraded products is examined by infrared micro-spectroscopy of the Amide I (protein) band. Changes similar to matrigel are observed in gelatin degradation. By removing the degraded gelatin from the surface and performing gel electrophoresis, smaller fragments of gelatin are observed. These observations suggest an unwinding of the gelatin fiber and subsequent cleavage.

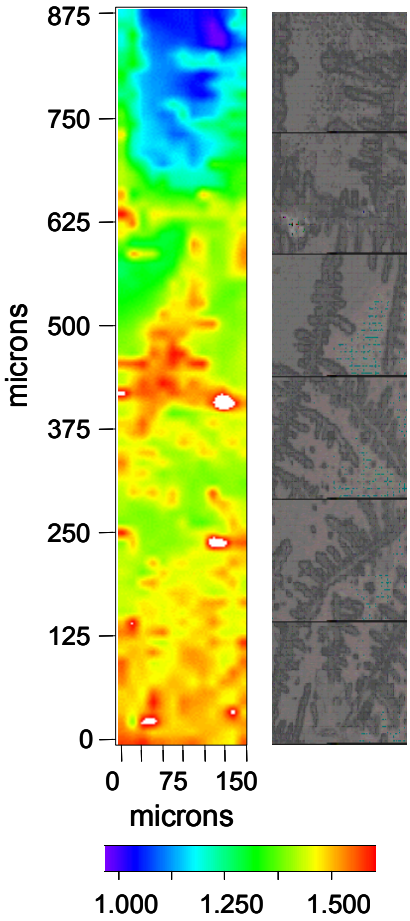

Figure 1. (Right) Visible and (Left) infrared image of partially degraded matrigel fibers on a BaF2 disk. The gelatinase B enzyme was deposited near the top of the IR/visible image. The infrared image was generated by calculating the ratio of the undegraded Amide I band (1660 cm^{-1}) and a degradation product peak at 1590 cm^{-1}. Thus, the higher ratio indicates undegraded matrigel.